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SPOTLIGHT

Microtubules keep large cells in shape

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Migrating cells need to coordinate extension and retraction of their protrusions to avoid fragmenting. Kopf et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201907154) demonstrate that microtubules help to maintain cell coherence during amoeboid migration by controlling actomyosin contractility in retracting protrusions.

The extension and retraction of protrusions in migrating cells is dependent on localized actin polymerization and actomyosin contractility; however, how these processes are coordinated at the level of the whole cell is still poorly understood. Such global regulation appears particularly challenging for large cells undergoing ameboid migration, such as dendritic cells (DCs). When DCs navigate through geometrically complex environments, they do not degrade the extracellular matrix but rather form multiple extended protrusions to explore their surroundings and choose the path of least resistance. To systematically study this process, Kopf et al. (1) exposed DCs to a chemokine gradient inside microfluidic devices, forcing the cells to choose between channels of different sizes and geometries. Using this setup, the same laboratory has previously shown that DCs use their nucleus as a mechanical gauge to probe pore size and search for an optimal passage (2). Once the nucleus and cell body translocate into a certain direction, other protrusions are retracted, but how is this communication between different cell protrusions achieved? In small leukocytes, such as neutrophils, this process is thought to be controlled by membrane tension, which in turn can regulate actomyosin dynamics (3). In adherent cells, cell coherence can depend on components of the actomyosin network, such as stress fibers (4). However, DCs lack stress fibers and are too large and ramified to allow equilibration of membrane tension, suggesting that another mechanism is likely to be at work. An interesting candidate for mediating the communication between protrusions is the microtubule cytoskeleton because its disassembly with nocodazole caused uncoordinated protrusions and fragmentation of DCs navigating multichannel spaces (2).

In this issue, Kopf et al. (1) explore the function of microtubules in this cell migration model in detail. In amoeboid cells, microtubules form a radial system that is anchored at the centrosome, which is typically positioned behind the nucleus (2). Similar to many other types of migrating cells (5), microtubules directed to the front of a migrating DC were more long-lived than those directed to the rear (1). Interestingly, when a cell had to choose between two channels and formed two front-facing protrusions, the "winning protrusion" was determined by the positioning of the microtubule-organizing center (2). The microtubule density remained high in this protrusion, while the "losing protrusion" was gradually emptied of microtubules and retracted (Fig. 1 A). To prove causality between microtubule polymerization and protrusion fate, the authors locally induced microtubule disassembly using photostatin PST-1, a photoswitchable microtubule-depolymerizing agent (6), and showed that local inhibition of microtubule growth leads to retraction of the illuminated protrusion. These data are consistent with previous experiments showing that local optogenetics-based inhibition of microtubule-stabilizing tip-associated complexes can steer cell movement (7).

Previous work has established that microtubule disassembly causes an increase in actomyosin contractility due to the release and activation of the microtubule-associated guanine nucleotide exchange factor of RhoA GTPase, GEF-H1/Lfc (8). Indeed, Kopf et al. (1) found that Lfc knockout cells displayed protrusion retraction defects and cell fragmentation in multifluidic devices, similar to nocodazole-treated cells. Lfc and its downstream target, myosin light chain (MLC), were enriched in trailing protrusions, and the loss of Lfc inhibited the peripheral localization of MLC (Fig. 1 A). Loss of Lfc in DCs also caused cell over-elongation on adhesive substrates, suggesting that Lfc regulates adhesion disassembly. This effect was exacerbated by pharmacological microtubule depolymerization, in line with the view that microtubules can regulate adhesion turnover through different pathways (9).

Importantly, the impact of manipulating microtubule density and actomyosin contractility was dependent on the geometry of the environment in which a cell was migrating. In one-dimensional channels, DCs lacking microtubules could move normally but switched directions more frequently, and this defect could be suppressed by inhibiting the Rho-associated protein kinase ROCK. However, this rescue did not work in complex multichannel environments, where ROCK inhibition alone was sufficient

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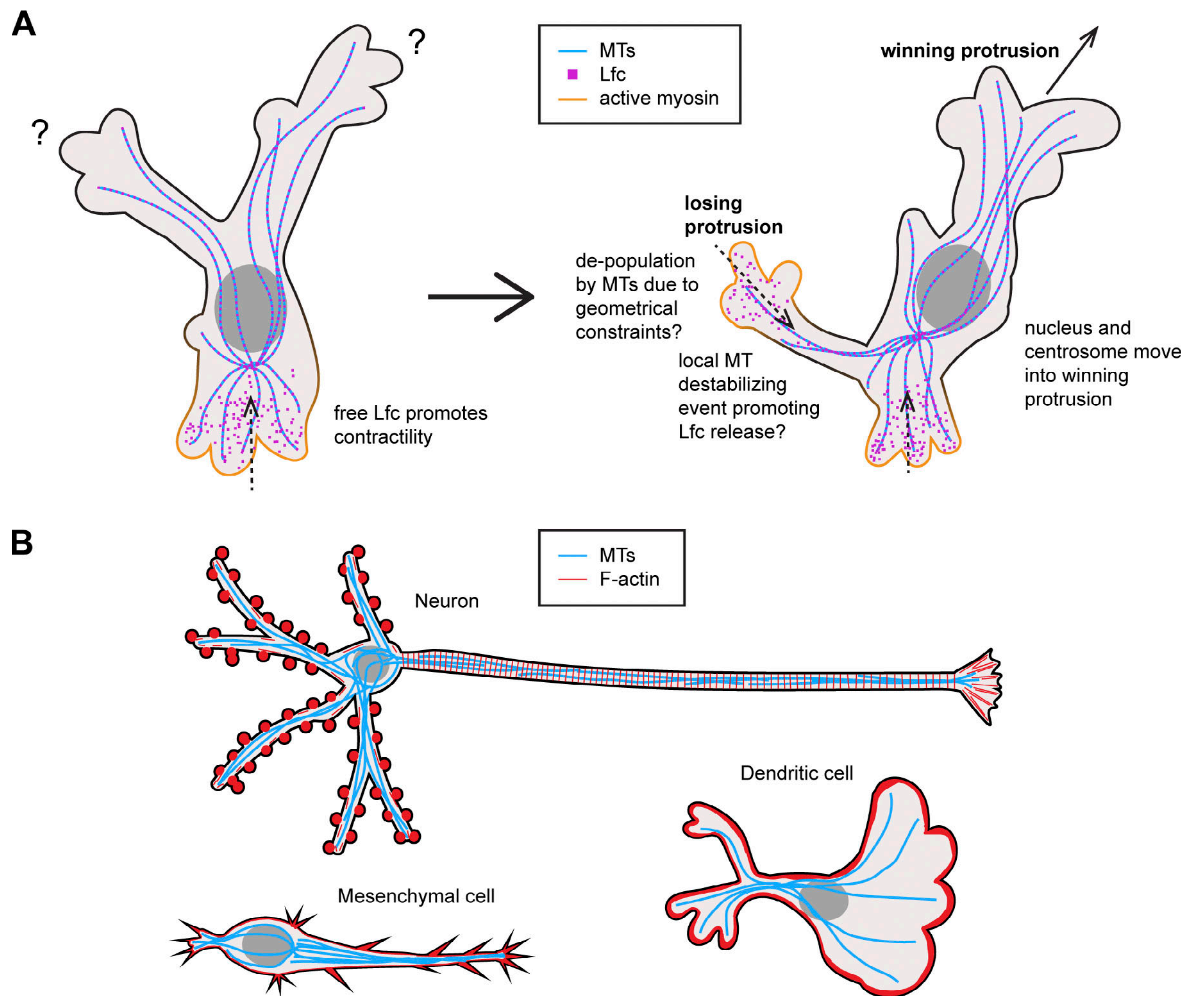

Figure 1. **Cytoskeletal organization in cell protrusions. (A)** A DC making a choice between two channels. The "winning" protrusion, which acquires the nucleus and the microtubule-organizing center, maintains high microtubule density, whereas in the "losing" protrusion, microtubules depolymerize, and this leads to the release of Lfc and activation of actomyosin contractility. **(B)** Organization of microtubules and actin in different types of large cells.

to cause cell entanglement. These observations emphasize the importance of microtubules and well-coordinated regulation of contractility for maintaining coherence of large cells with complex geometries.

This work supports the critical role of microtubules for maintaining complex shapes of large cells. Cells with the longest protrusions in our body, neurons, strongly depend on microtubules for their overall architecture (Fig. 1 B). Mesenchymal cells, such as fibroblasts and endothelial and cancer cells, can maintain their shape on hard two-dimensional substrates in the absence of microtubules but require microtubules for forming long protrusions in soft environments (5, 10; Fig. 1 B). In these cell types, microtubules typically form dense arrays and can contribute to cell morphology by controlling cell mechanics as well as signaling and trafficking (5). In contrast, amoeboid cells lack dense microtubule networks, and the actomyosin system and membrane tension appear to be sufficient to control their shape when they are small. However, large and ramified amoeboid cells, such as DCs, do require microtubules for controlling their morphology (Fig. 1 B). As shown by Kopf et al. (1), this occurs in part through signaling; however, additional mechanisms associated with microtubule-based transport are likely to be involved.

The observations described by Kopf et al. raise many interesting questions. What controls microtubule polarity in DCs? In mesenchymal cells and in neurons, different microtubule plus end–tracking proteins, such as CLASPs, APC, and spectraplakins, have been implicated, but it is unknown which factors perform this function in amoeboid cells. What governs the asymmetric distribution and activation of Lfc that preferentially accumulates in the cell rear (1)? Is Lfc distribution controlled by signaling mediators that respond to the overall polarity in DCs? Does the differential stability of microtubules at the leading edge and at the rear play a role in Lfc localization? What is the mechanism promoting microtubule depolymerization in retracting protrusions? The authors propose that when the cell nucleus and the centrosome move into one of the protrusions, the other ones could be cut off from the supply of new microtubules simply due to geometrical constraints: growing microtubules are too rigid to form sharp turns and cannot efficiently penetrate into curved spaces. It is conceivable that dynamic microtubules could regulate their own longevity and

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density though feedback mechanisms; for example, growing microtubules could stimulate a microtubule-stabilizing signaling pathway, whereas reduction in their number could provide a signal to promote local microtubule disassembly. Detailed understanding of the molecular pathways controlling microtubule organization will enable answering these questions and may help to generate a unified view of cytoskeletal control of cell shape. Given that microtubules remain major targets for treatment of cancer and certain inflammatory conditions, such understanding may contribute to the development of improved therapies.

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